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Characterization of the Binding Surface of the Human Protein GABARAP

Yvonne Thielmann¹, Oliver H. Weiergräber¹, Jeannine Mohrlüder^{1,2},
Bernd W. König^{1,2}, Rudolf Hartmann¹, Thomas Stangler^{1,2},
Katja Wiesehan¹, and Dieter Willbold^{1,2}

¹ Institut für Neurowissenschaften und Biophysik, Research Centre Jülich, 52425 Jülich, Germany
E-mail: {y.thielmann, d.willbold}@fz-juelich.de

² Institut für Physikalische Biologie und BMFZ, Heinrich-Heine-Universität,
40225 Düsseldorf, Germany

GABA(A) receptors are ligand-gated chloride channels that mediate inhibitory neurotransmission. The GABA_A receptor associated protein (GABARAP) interacts with the gamma2 subunit of the GABA_A receptor, modulates channel kinetics and promotes receptor clustering. Two hydrophobic pockets acting as indole binding sites were identified as major determinants of the ligand specificity of GABARAP by two dimensional NMR. We identified peptide K1 that binds GABARAP with high affinity. Co-crystals of GABARAP and K1 diffract to 1.3 Å resolution. Each hydrophobic pocket of GABARAP is occupied by a tryptophan residue of the peptide. Recently we found that calreticulin binds GABARAP. Co-crystals of GABARAP and calreticulin (178-188) diffract to a resolution of 2.3 Å. In this case the two hydrophobic pockets are occupied by a tryptophan and a leucine, respectively. This is the first complex structure of GABARAP with a native ligand.

1 GABARAP Displays Two Hydrophobic Pockets

The role of tryptophan as a key residue for ligand binding to the ubiquitin-like modifier GABARAP was investigated. Two tryptophan binding hydrophobic patches were identified on the conserved face of the GABARAP structure by NMR spectroscopy and molecular docking (Figure 1). GABARAP binding of indole and indole derivatives including the free amino acid tryptophan was quantified. The two tryptophan binding sites can be clearly distinguished by mapping the NMR-derived residue specific apparent dissociation constant, K_d , onto the three-dimensional structure of GABARAP. The biological relevance of tryptophan binding pockets of GABARAP is supported by a highly conserved tryptophan residue in the GABARAP binding region of calreticulin, clathrin heavy chain, and the gamma2 subunit of the GABA_A receptor. Replacement of tryptophan by alanine abolishes ligand binding to GABARAP¹.

2 Co-Crystallization of GABARAP with Ligand Peptides

Next we have determined the X-ray structure of the soluble form of human GABARAP complexed with a high-affinity synthetic peptide at 1.3 Å resolution (Figure 2). The data shed light on the probable binding modes of key interaction partners, including the GABA_A receptor and the cysteine protease Atg4. The resulting models provide a structural background for further investigation of the unique biological properties of GABARAP².

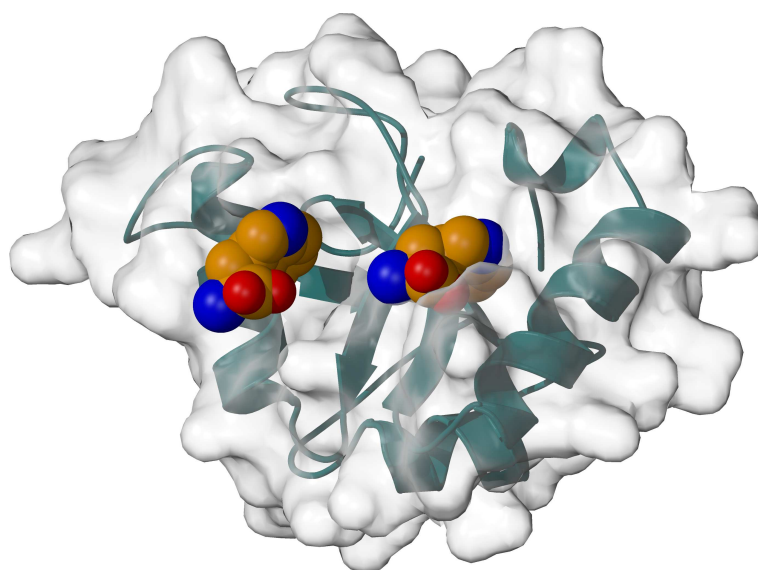


Figure 1. GABARAP in ribbon and surface representation. Two indole molecules are placed in the hydrophobic pockets HP1 and HP2, respectively.

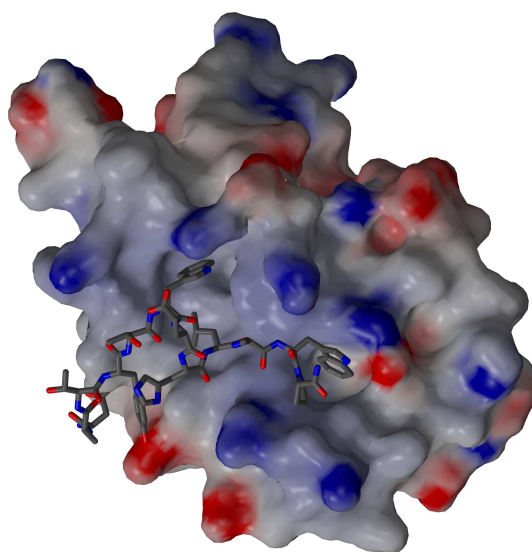


Figure 2. Overview of the GABARAP-K1 complex. GABARAP is depicted as a surface representation. The peptide is shown in stick mode in dark gray. Both tryptophans are deeply buried in the hydrophobic pockets.

Recently we have succeeded in co-crystallizing GABARAP and a fragment of calreticulin and have determined the X-ray structure at 2.3 Å resolution (Figure 3). The results

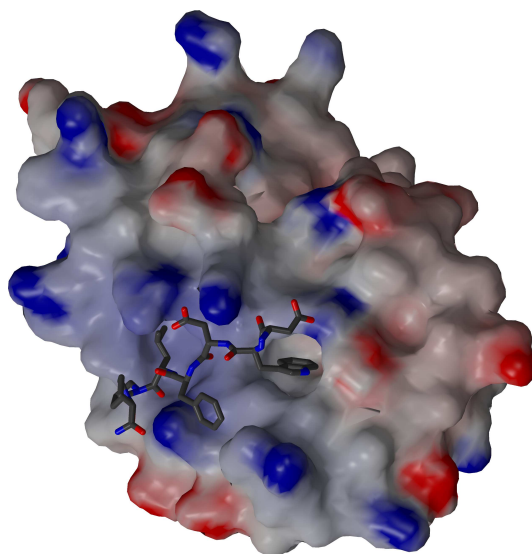


Figure 3. Overview of the GABARAP-CRT(178-188) complex. GABARAP is depicted as a surface representation. The peptide is shown in stick mode in dark gray. The tryptophan and leucine residues are buried in HP1 and HP2, respectively.

improve our understanding of the GABARAP-calreticulin interaction and serve as a model for the interaction with clathrin heavy chain and NSF. The derived model of the complex with full length calreticulin provides a starting point for the investigation of multimeric complexes with additional binding partners of GABARAP.

Acknowledgments

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